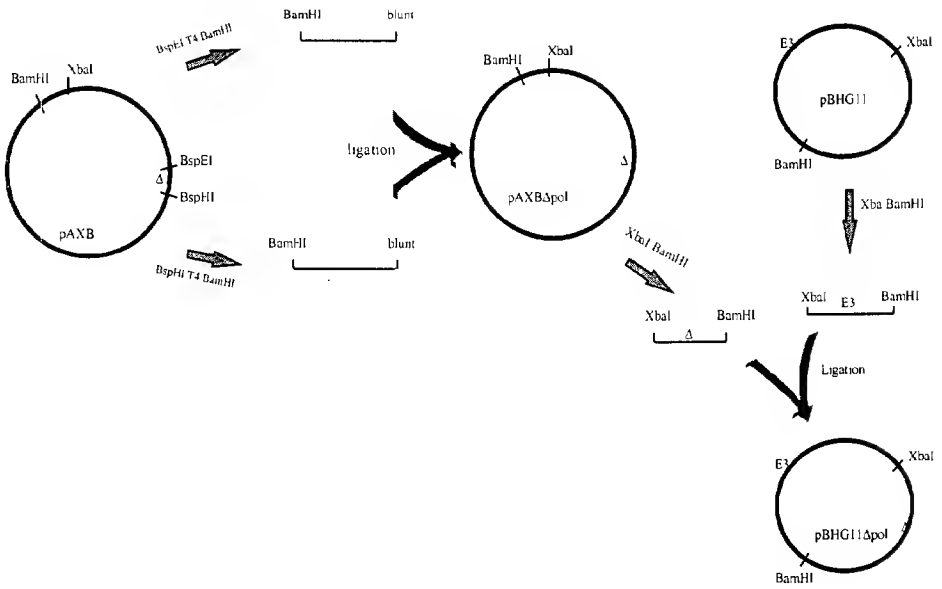

BamHI
XbaI
BspEI T4 BamHI
BspEI
BspHI
pAXB
BspHI T4 BamHI
BamHI
blunt
ligation
BamHI
XbaI
pAXBΔpol
BamHI
blunt
XbaI BamHI
XbaI
BamHI
E3
XbaI
pBHG11
BamHI
Xba BamHI
XbaI
E3
BamHI
Ligation
E3
XbaI
pBHG11Δpol
BamHI

Figure 1A

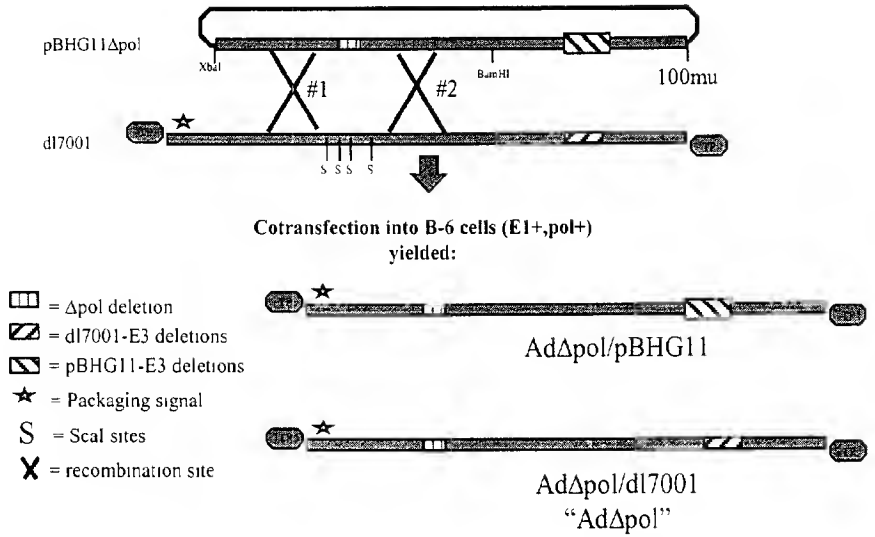

pBHG11Δpol
XbaI
BamHI
100mu
#1
#2
dl7001
S S S S
Cotransfection into B-6 cells (E1+,pol+)
yielded:
= Δpol deletion
= dl7001-E3 deletions
= pBHG11-E3 deletions
= Packaging signal
S = ScaI sites
= recombination site
AdΔpol/pBHG11
AdΔpol/dl7001
"AdΔpol"

Figure 1B

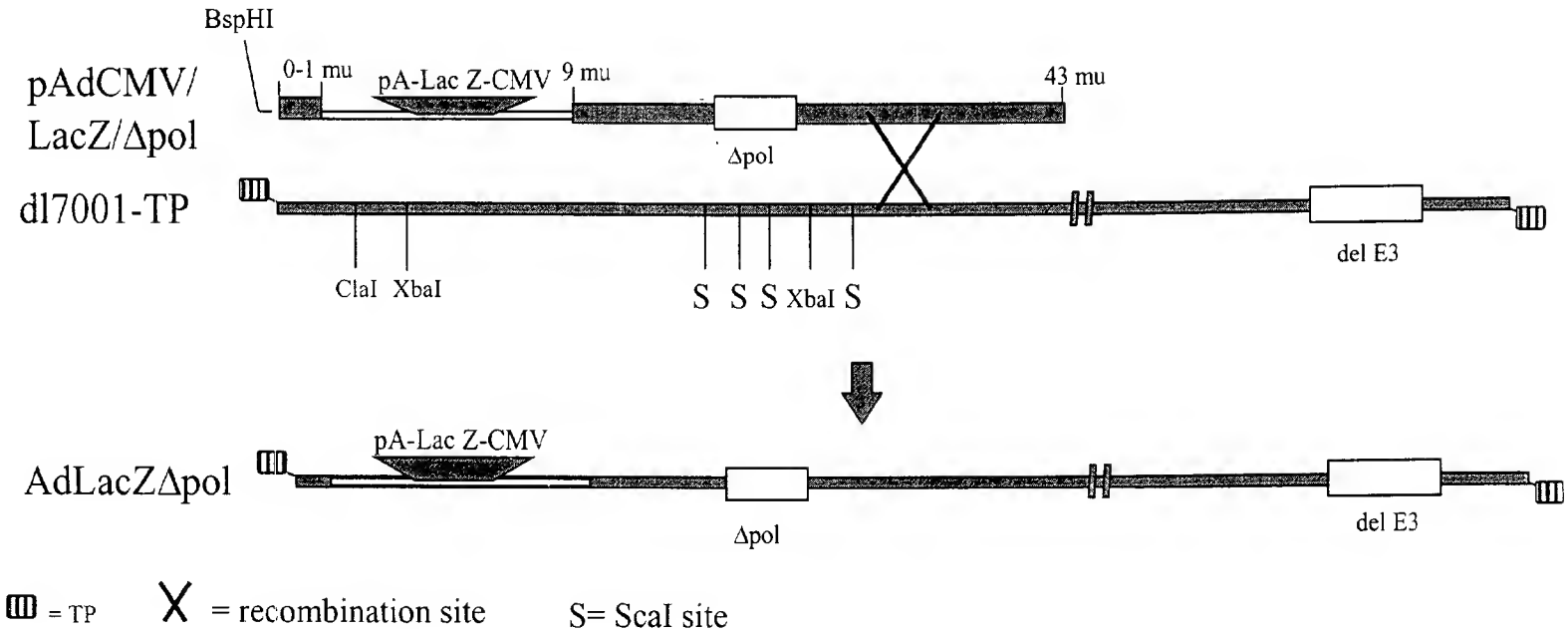

BspHI
pAdCMV/
LacZ/Δpol
0-1 mu
pA-Lac Z-CMV
9 mu
43 mu
Δpol
dl7001-TP
ClaI
XbaI
S
S
S
XbaI
S
del E3
pA-Lac Z-CMV
AdLacZΔpol
Δpol
del E3
= TP
= recombination site
S= ScaI site

Figure 1C

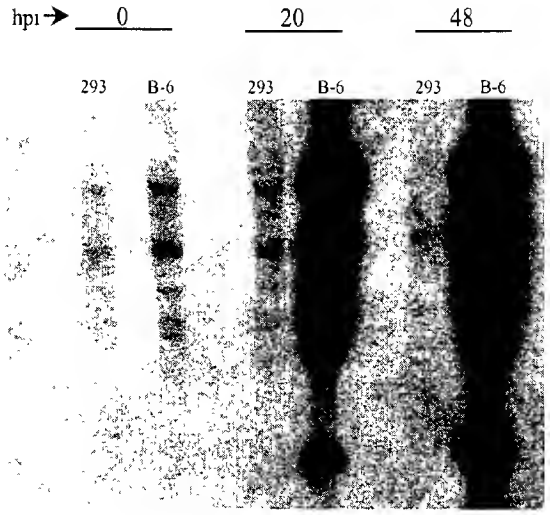


Figure 2

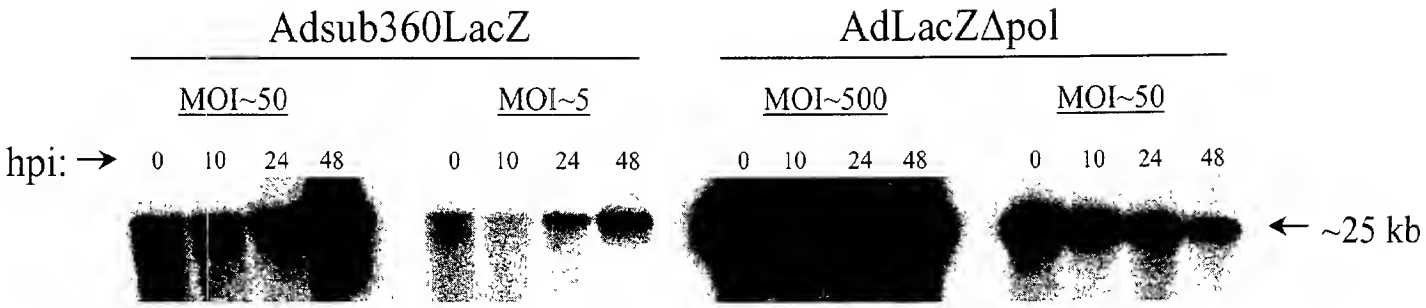

Adsub360LacZ
AdLacZΔpol
MOI~50
MOI~5
MOI~500
MOI~50
hpi:
0 10 24 48
0 10 24 48
0 10 24 48
0 10 24 48
~25 kb

Figure 3

Figure 4

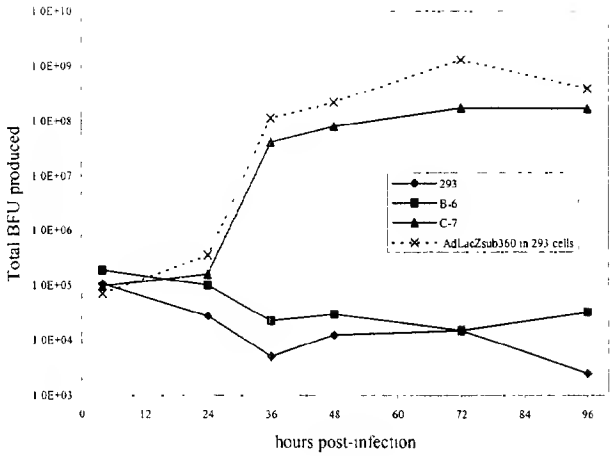

Total BFU produced
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
0
12
24
36
48
60
72
84
96
hours post-infection
293
B-6
C-7
AdLacZsub360 in 293 cells

Figure 5

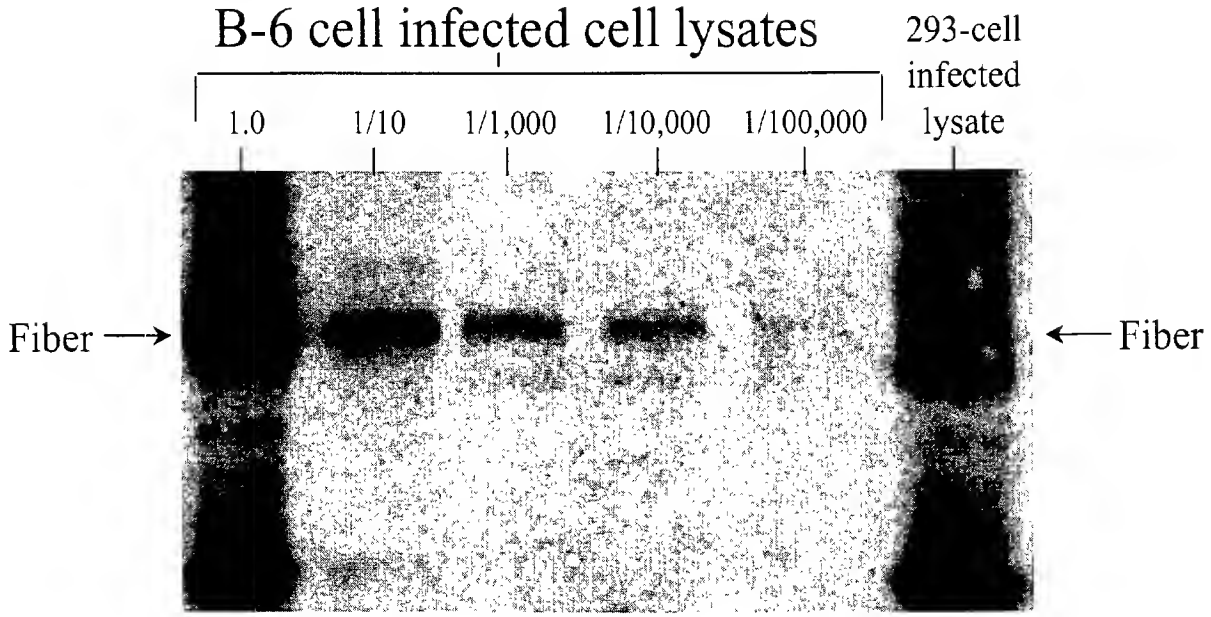

B-6 cell infected cell lysates
293-cell infected lysate
1.0
1/10
1/1,000
1/10,000
1/100,000
Fiber
Fiber

Figure 6

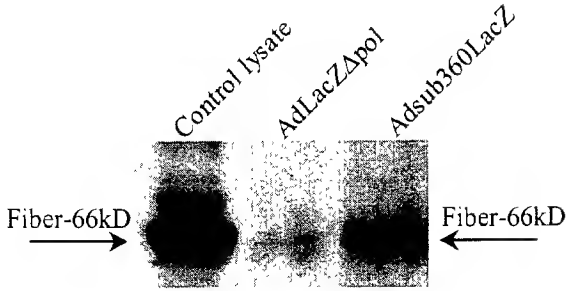

Control lysate
AdLacZΔpol
Adsub360LacZ
Fiber-66kD
Fiber-66kD

Figure 7

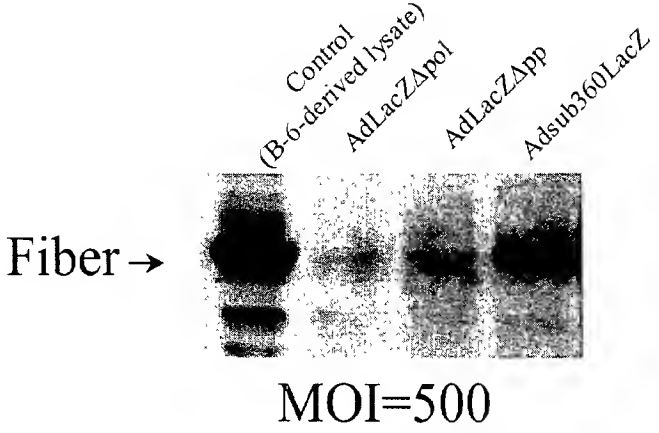

Control
(B-6-derived lysate)
AdLacZΔpol
AdLacZΔpp
Adsub360LacZ
Fiber →
MOI=500

Figure 8

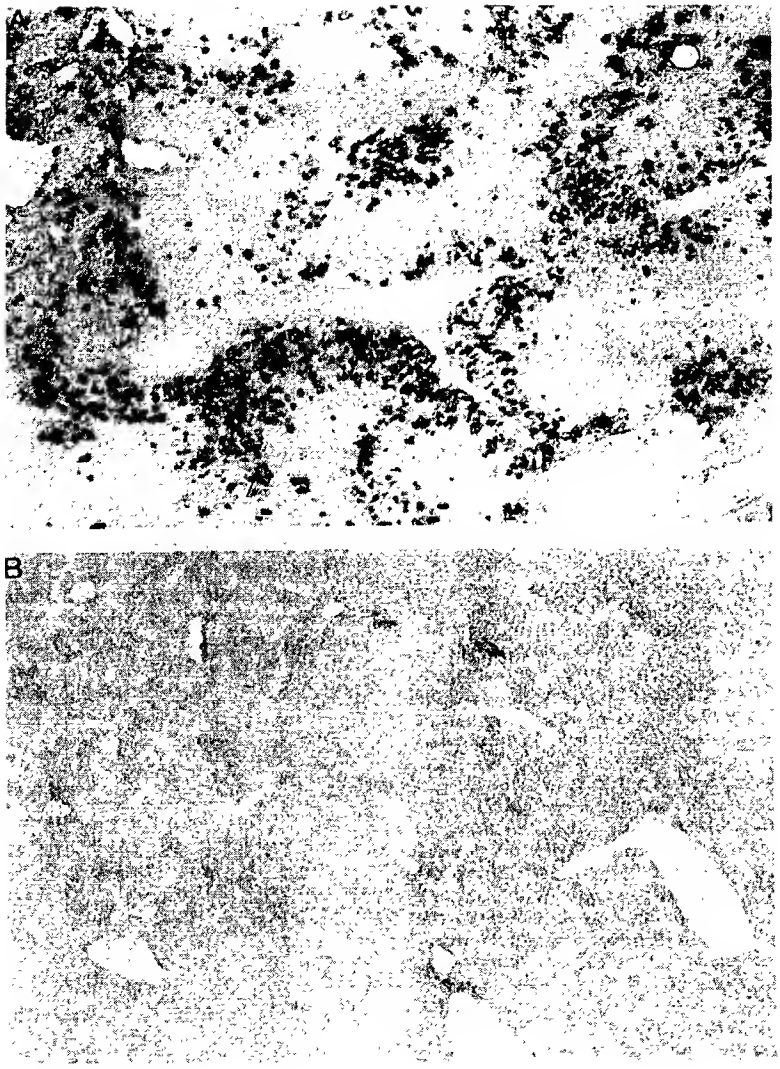


Figure 9

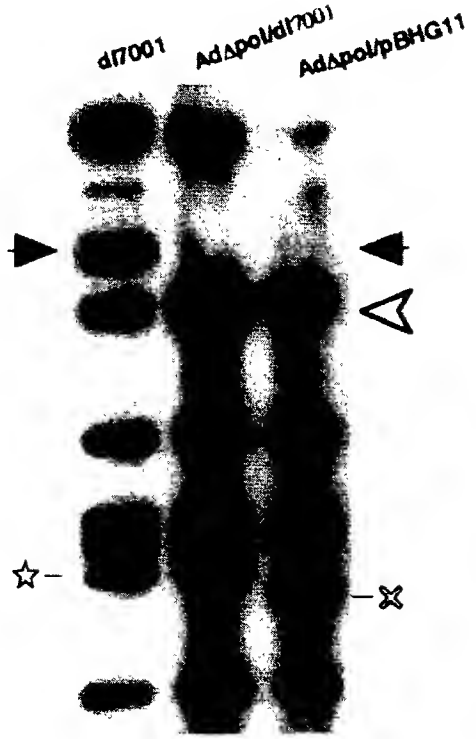


Figure 10

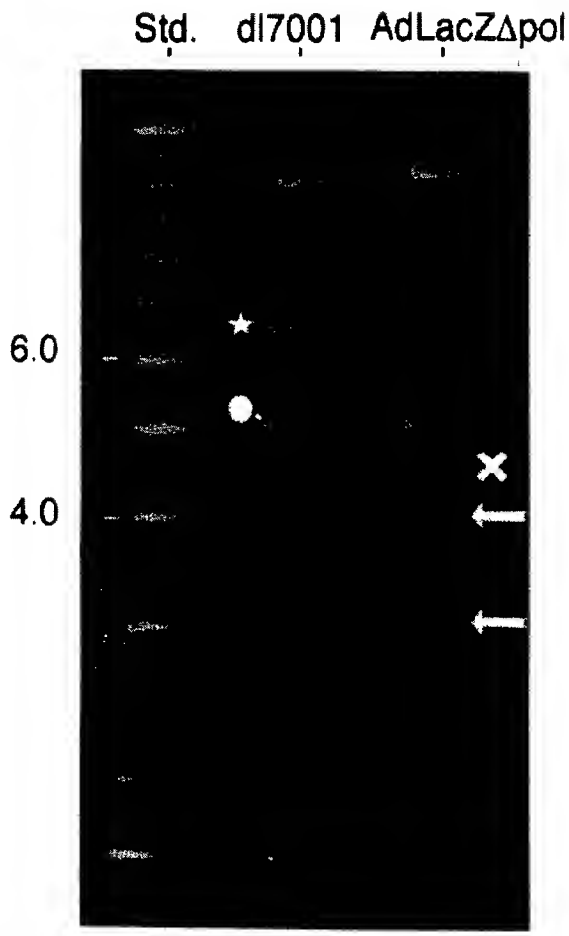


Figure 11

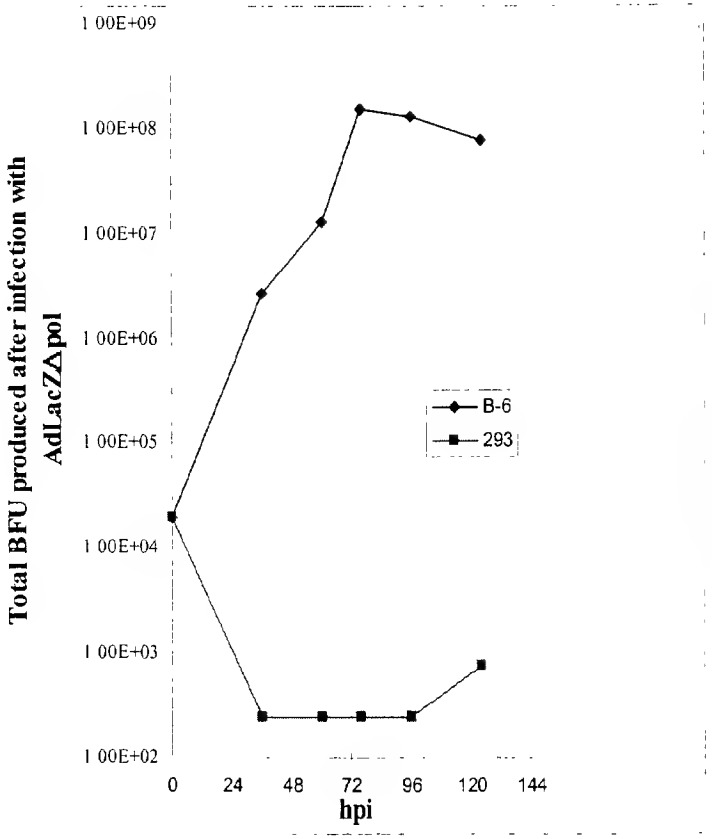

Total BFU produced after infection with AdLacZΔpol
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
0
24
48
72
96
120
144
hpi
B-6
293

Figure 12

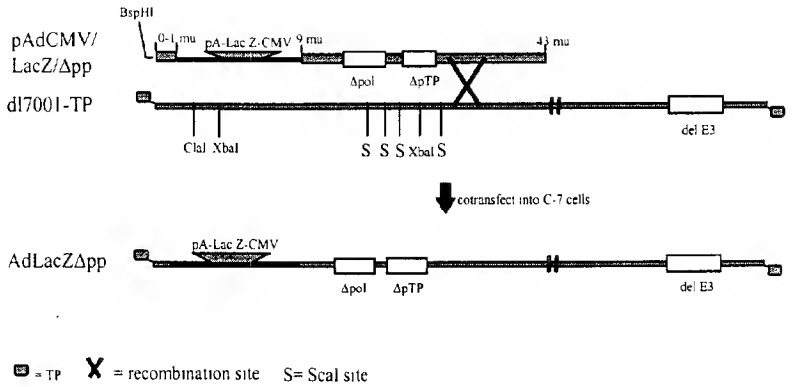

BspHI
pAdCMV/
LacZ/Δpp
0-1 mu
pA-Lac Z-CMV 9 mu
4.3 mu
Δpol
ΔpTP
dl7001-TP
del E3
ClaI XbaI
S S S XbaI S
cotransfect into C-7 cells
pA-Lac Z-CMV
AdLacZΔpp
Δpol
ΔpTP
del E3
= TP
= recombination site
S= ScaI site

Figure 13

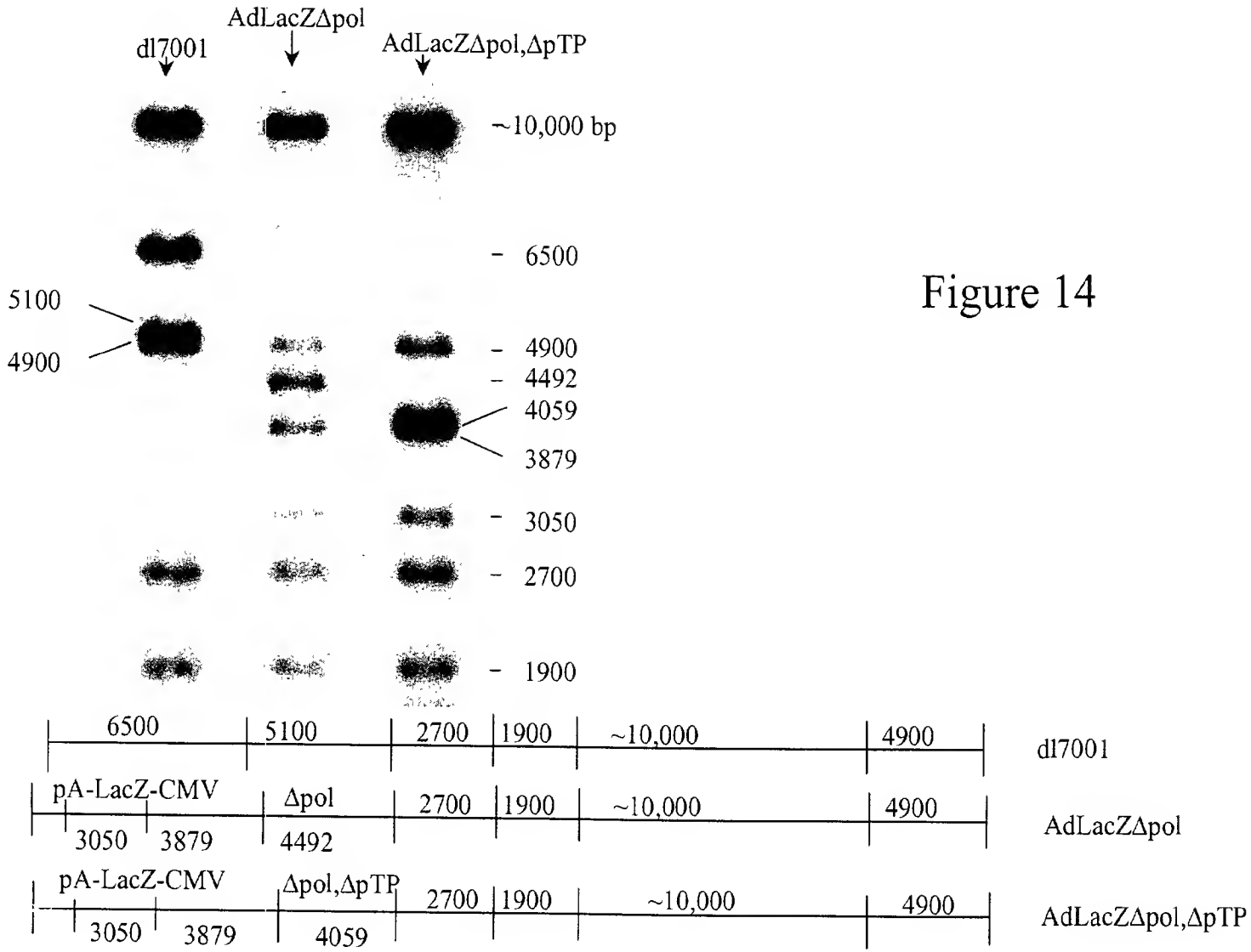

dl7001
AdLacZΔpol
AdLacZΔpol,ΔpTP
~10,000 bp
6500
5100
4900
4900
4492
4059
3879
3050
2700
1900
6500
5100
2700
1900
~10,000
4900
dl7001
pA-LacZ-CMV
Δpol
2700
1900
~10,000
4900
AdLacZΔpol
3050
3879
4492
pA-LacZ-CMV
ΔpolΔpTP
2700
1900
~10,000
4900
AdLacZΔpol,ΔpTP
3050
3879
4059

Figure 14

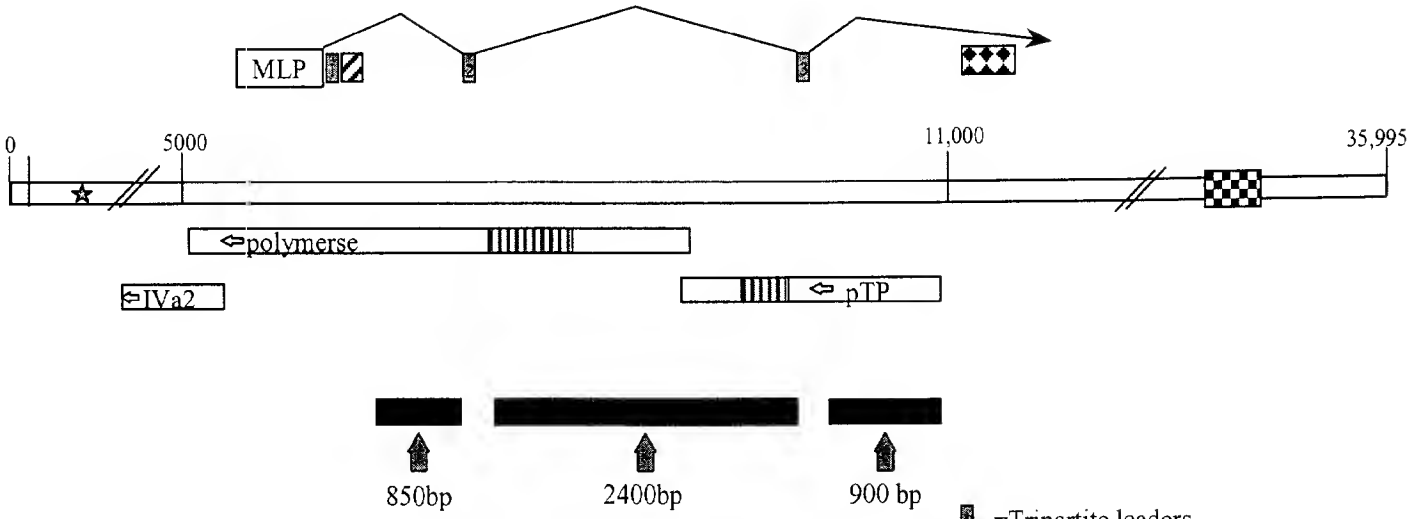

MLP
0
5000
11,000
35,995
polymerse
IVa2
pTP
850bp
2400bp
900 bp
=Tripartite leaders
=MLP-intron 1
=transgene insertion/ E1 deletion site
=VA RNA genes
=dl7001-E3 deletion
=del pol and del pTP deletions
present in AdLacZΔpp

Figure 15

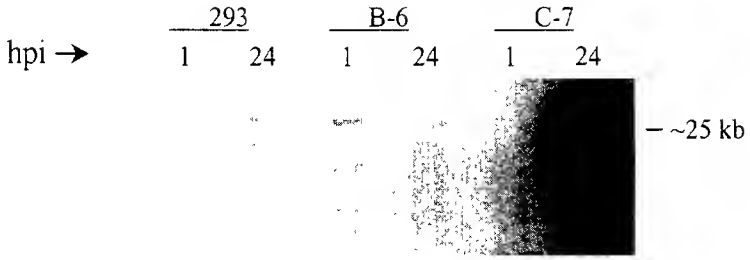


Figure 16

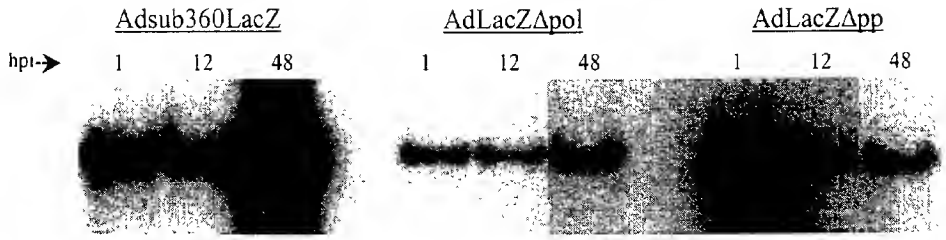


Figure 17

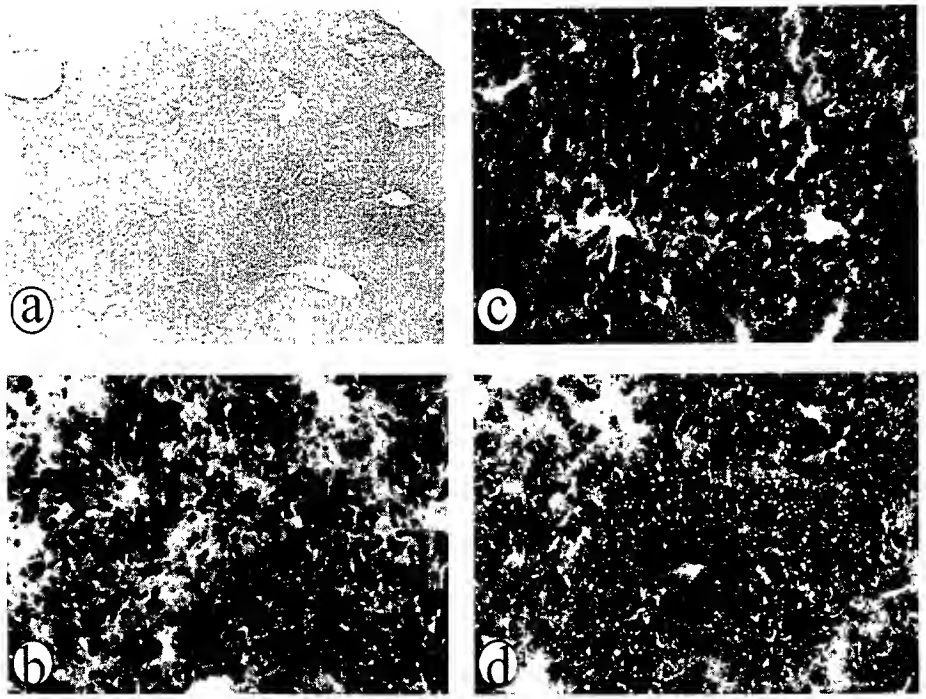
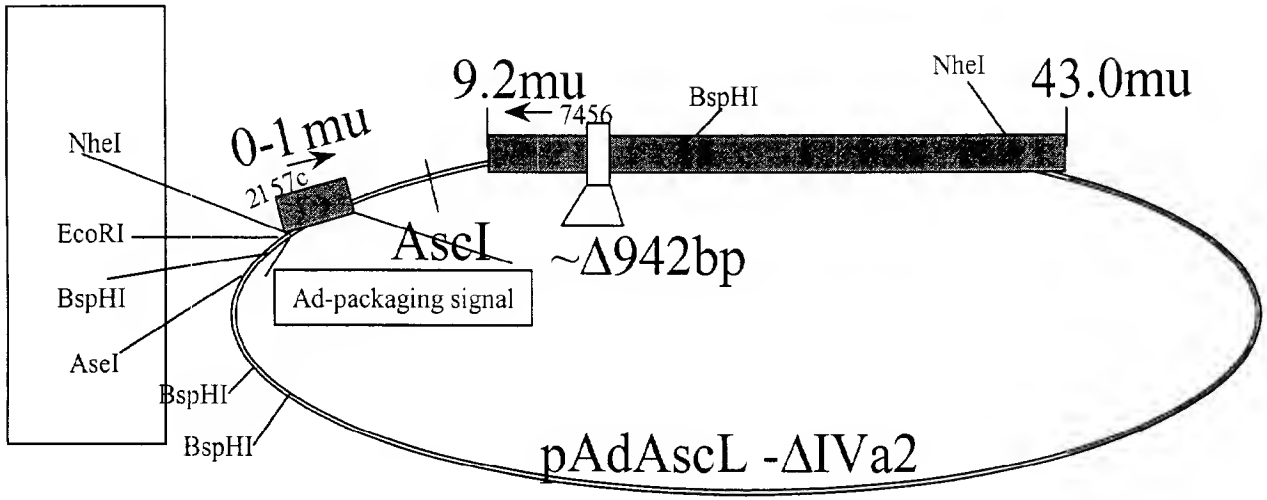


Figure 18

pAdAscL-ΔIVa2 shuttle plasmid(~14.2kb) Amp-Res

Linearization sites



← primer site location of indicated primer

Figure 19

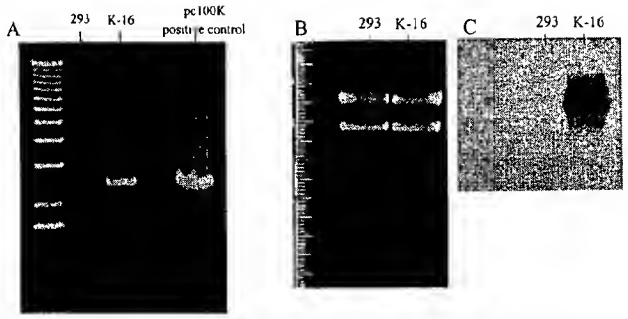
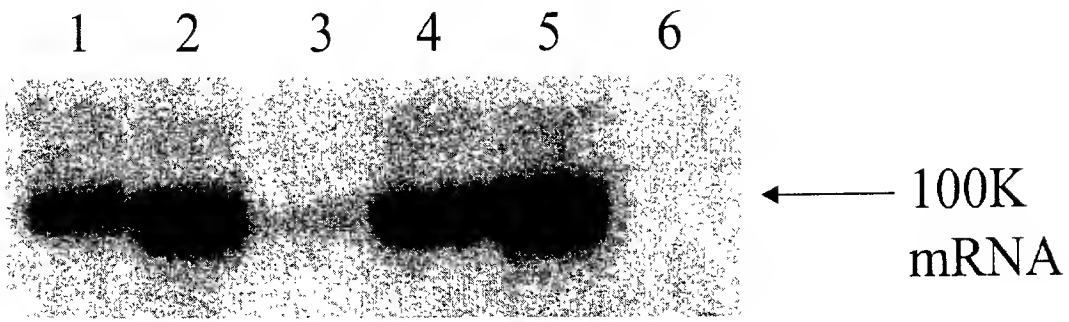


Figure 20



E1+pol+pTP+IVa2+100K= Lanes 1-4

E1+100K=Lane 5

E1= Lane 6

Figure 21

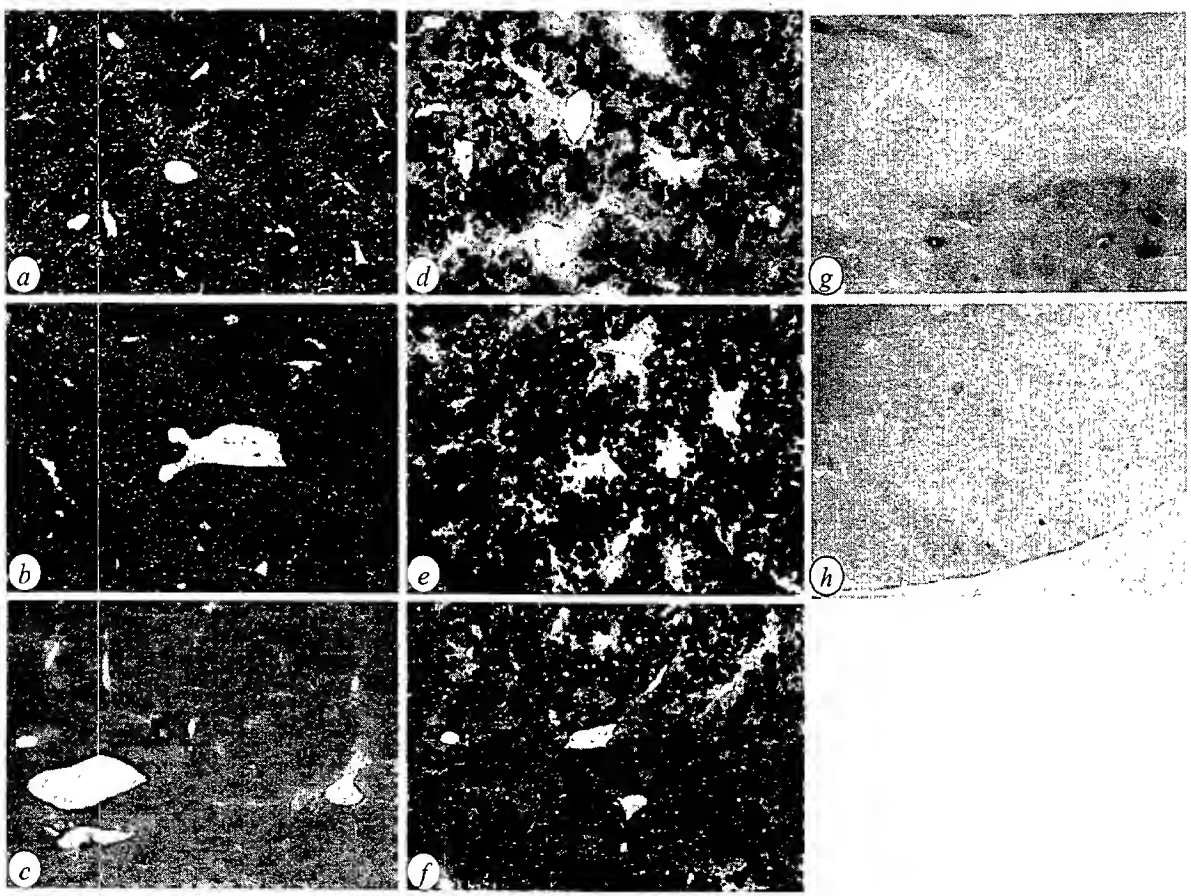


Figure 22

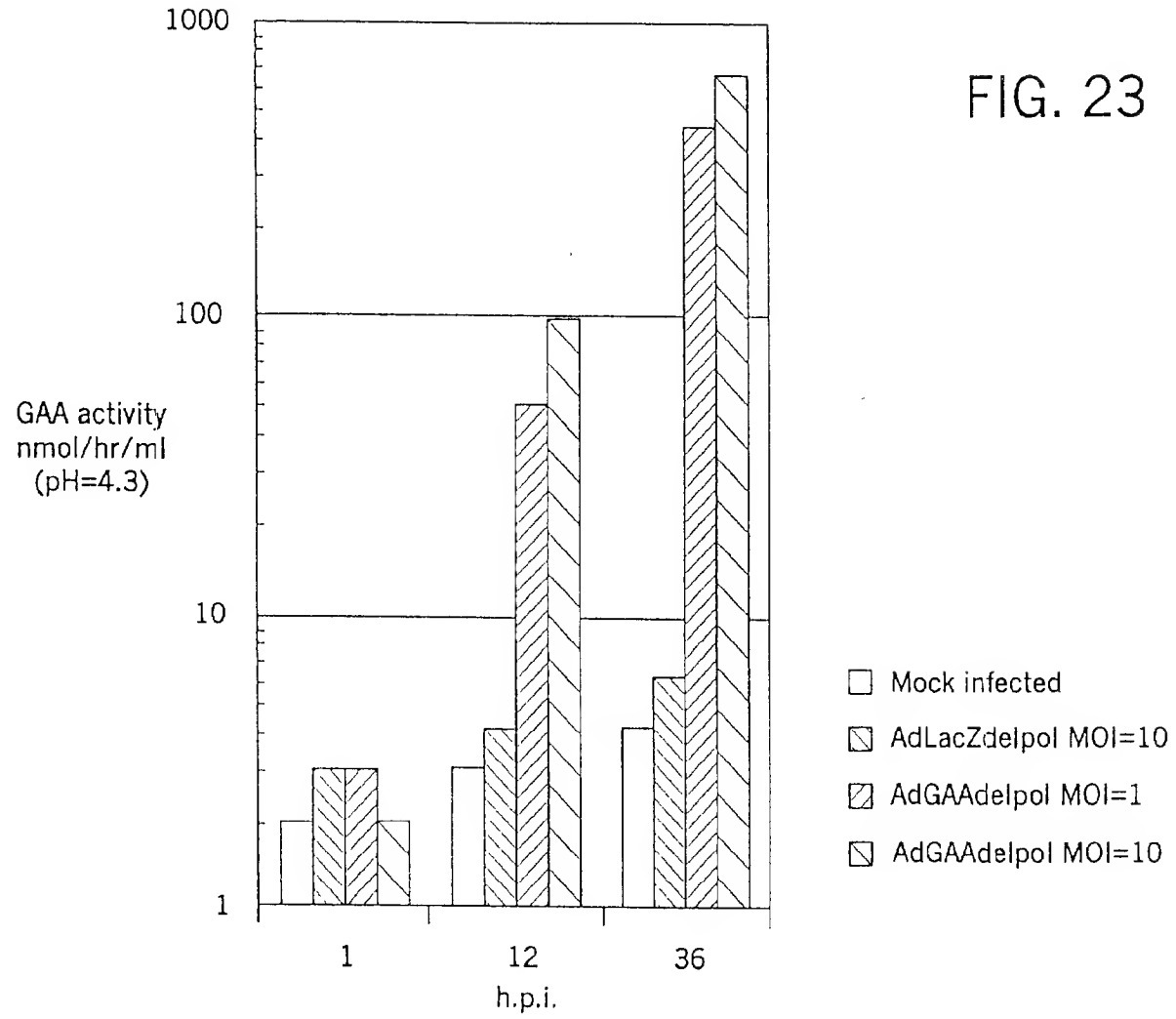

1000
100
10
1
GAA activity
nmol/hr/ml
(pH=4.3)
1
12
36
h.p.i.
Mock infected
AdLacZdelpol MOI=10
AdGAAdelpol MOI=1
AdGAAdelpol MOI=10

FIG. 23

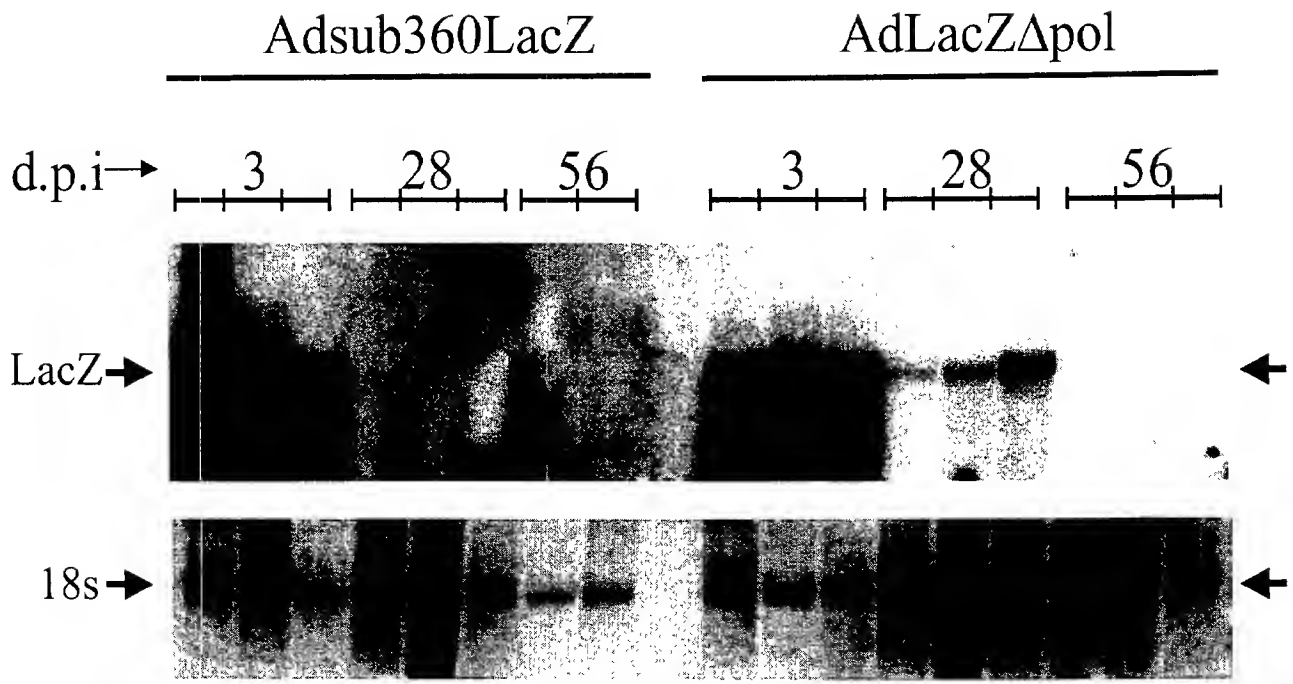


Figure 24

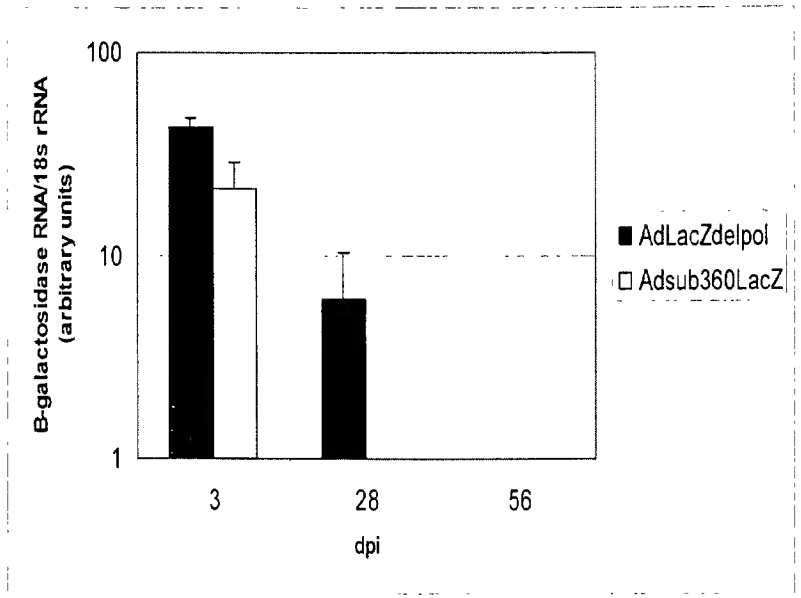

100
10
1
B-galactosidase RNA/18s rRNA
(arbitrary units)
3
28
56
dpi
AdLacZdelpol
Adsub360LacZ

Figure 25

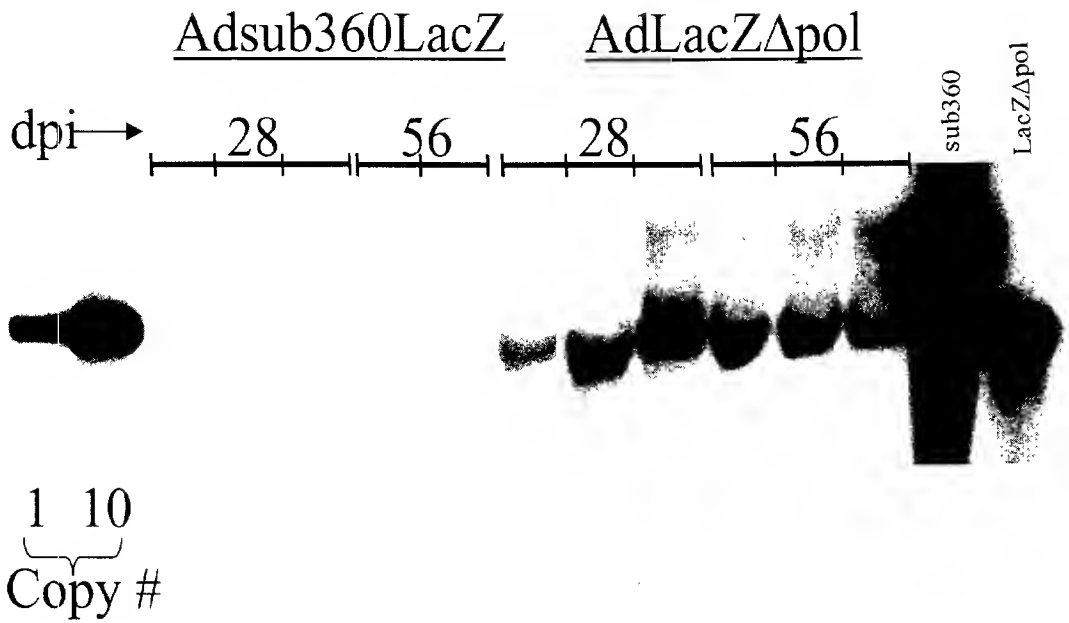

Adsub360LacZ
AdLacZΔpol
dpi
28
56
28
56
sub360
LacZΔpol
1 10
Copy #

Figure 26

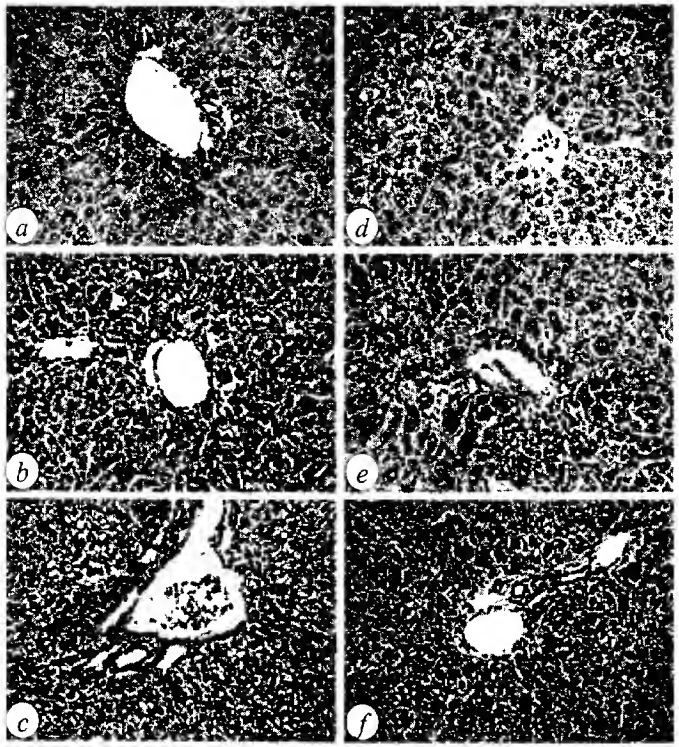


Figure 27

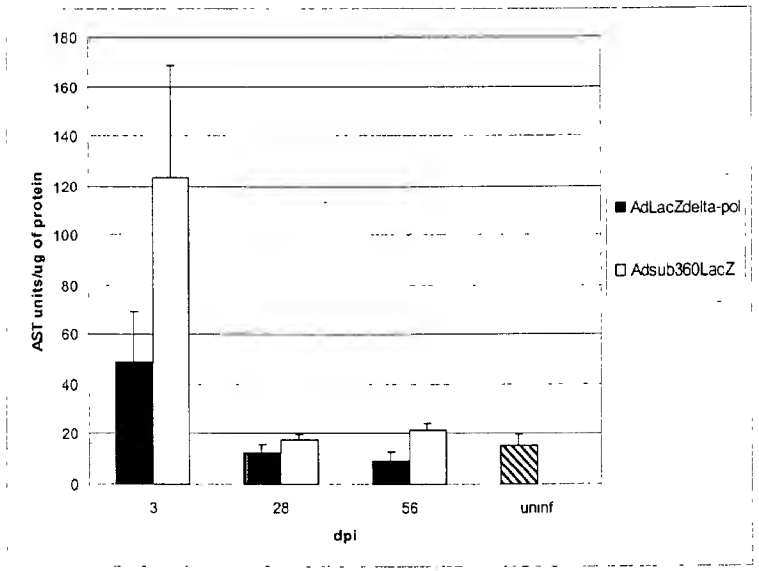

180
160
140
120
100
80
60
40
20
0
AST units/ug of protein
3
28
56
uninf
dpi
AdLacZdelta-pol
Adsub360LacZ

Figure 28

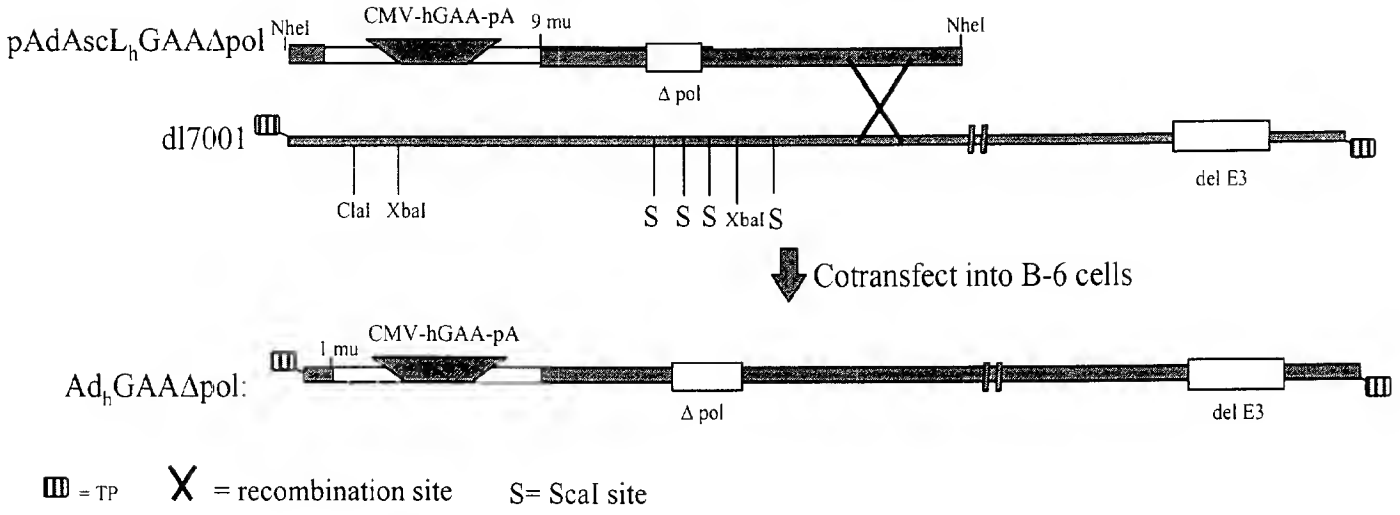

pAdAscL$_h$GAAΔpol
NheI
CMV-hGAA-pA
9 mu
NheI
Δ pol
dl7001
del E3
ClaI
XbaI
S S S XbaI S
Cotransfect into B-6 cells
Ad$_h$GAAΔpol:
1 mu
CMV-hGAA-pA
Δ pol
del E3
= TP
= recombination site
S= ScaI site

Figure 29

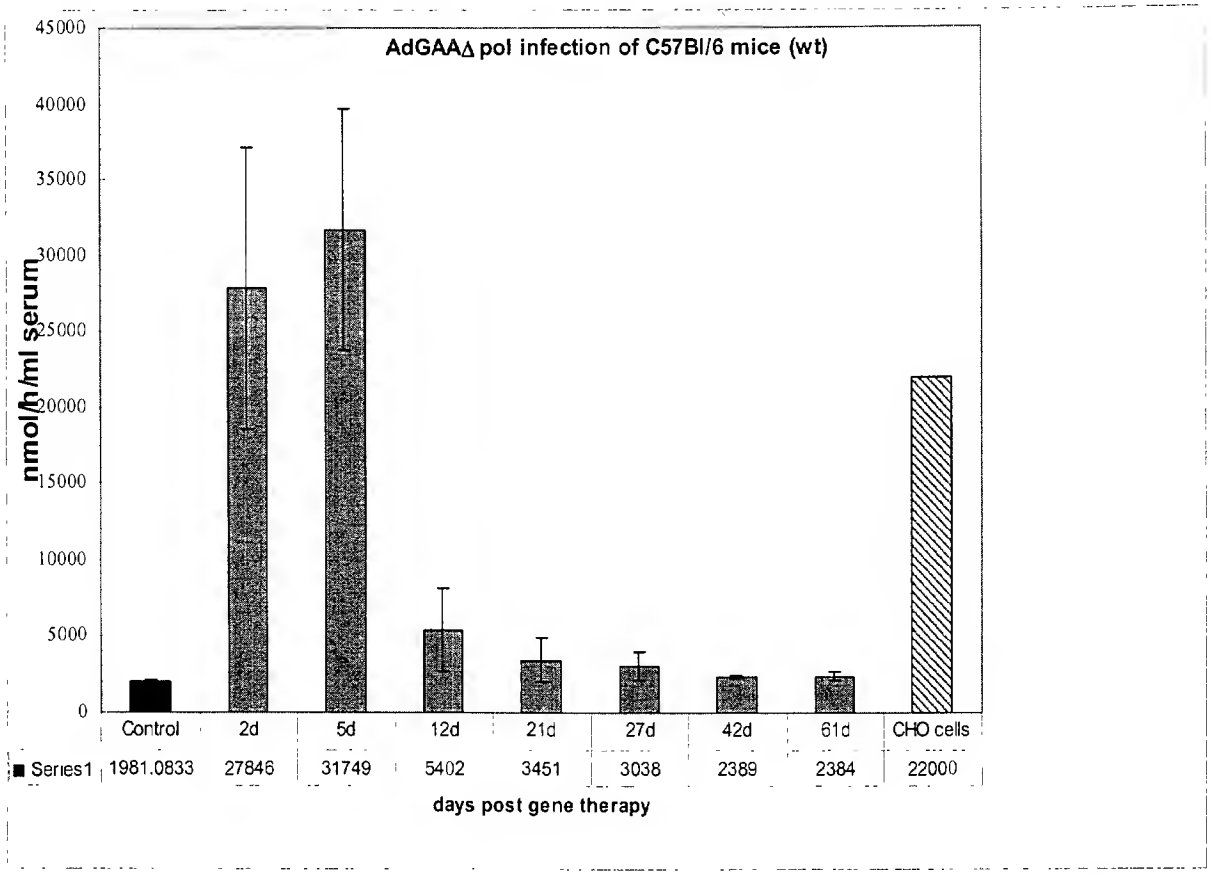

AdGAAΔ pol infection of C57Bl/6 mice (wt)
nmol/h/ml serum
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Control
2d
5d
12d
21d
27d
42d
61d
CHO cells
Series1
1981.0833
27846
31749
5402
3451
3038
2389
2384
22000
days post gene therapy

Figure 31

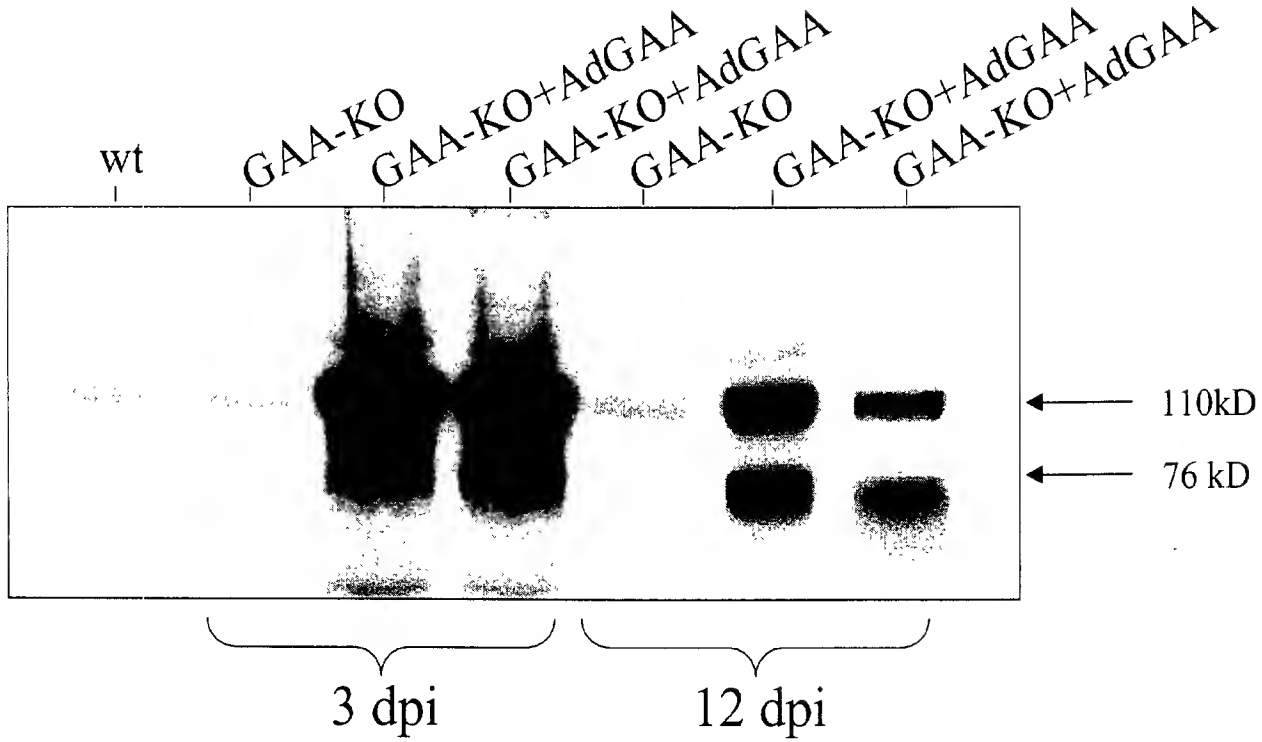

wt
GAA-KO
GAA-KO+AdGAA
GAA-KO+AdGAA
GAA-KO
GAA-KO+AdGAA
GAA-KO+AdGAA
110kD
76 kD
3 dpi
12 dpi

Figure 32

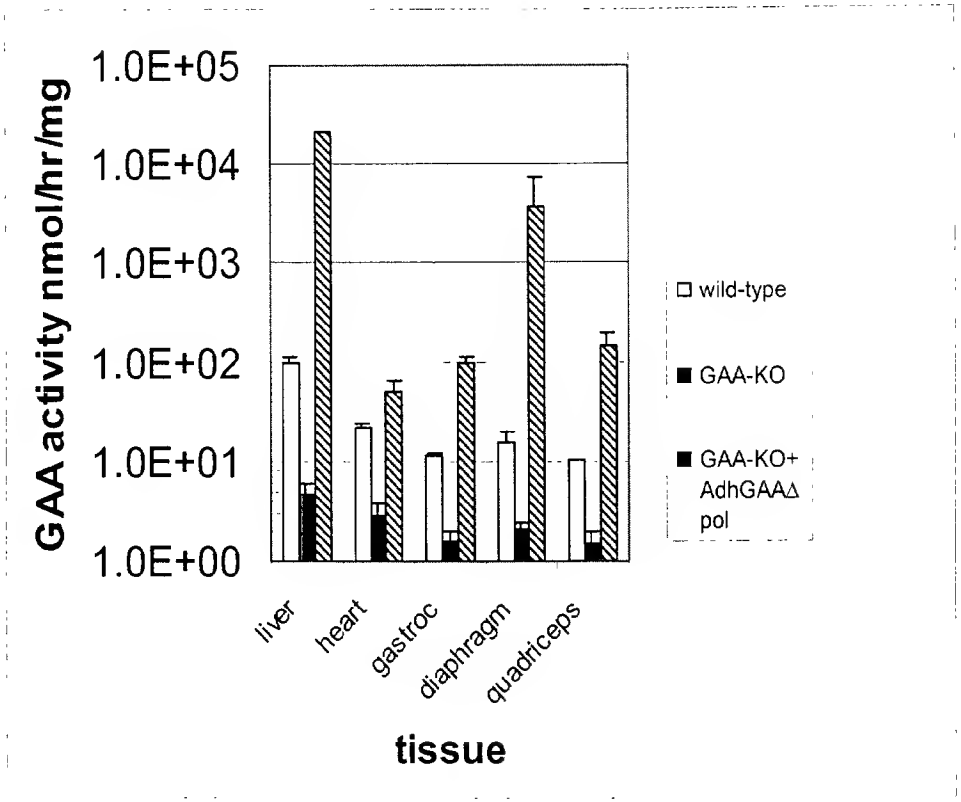

GAA activity nmol/hr/mg
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
liver
heart
gastroc
diaphragm
quadriceps
tissue
wild-type
GAA-KO
GAA-KO+ AdhGAAΔ pol

Figure 33

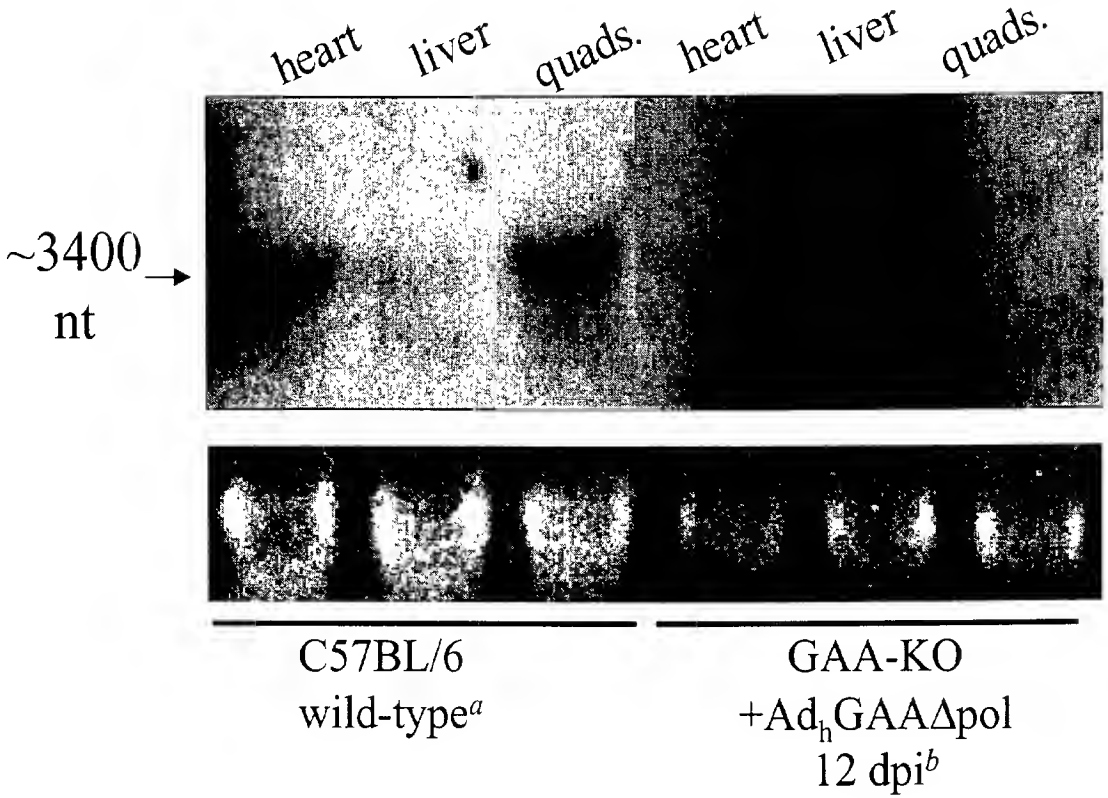

heart
liver
quads.
heart
liver
quads.
~3400
nt
C57BL/6
wild-type[a]
GAA-KO
+Ad$_h$GAAΔpol
12 dpi[b]

Figure 34

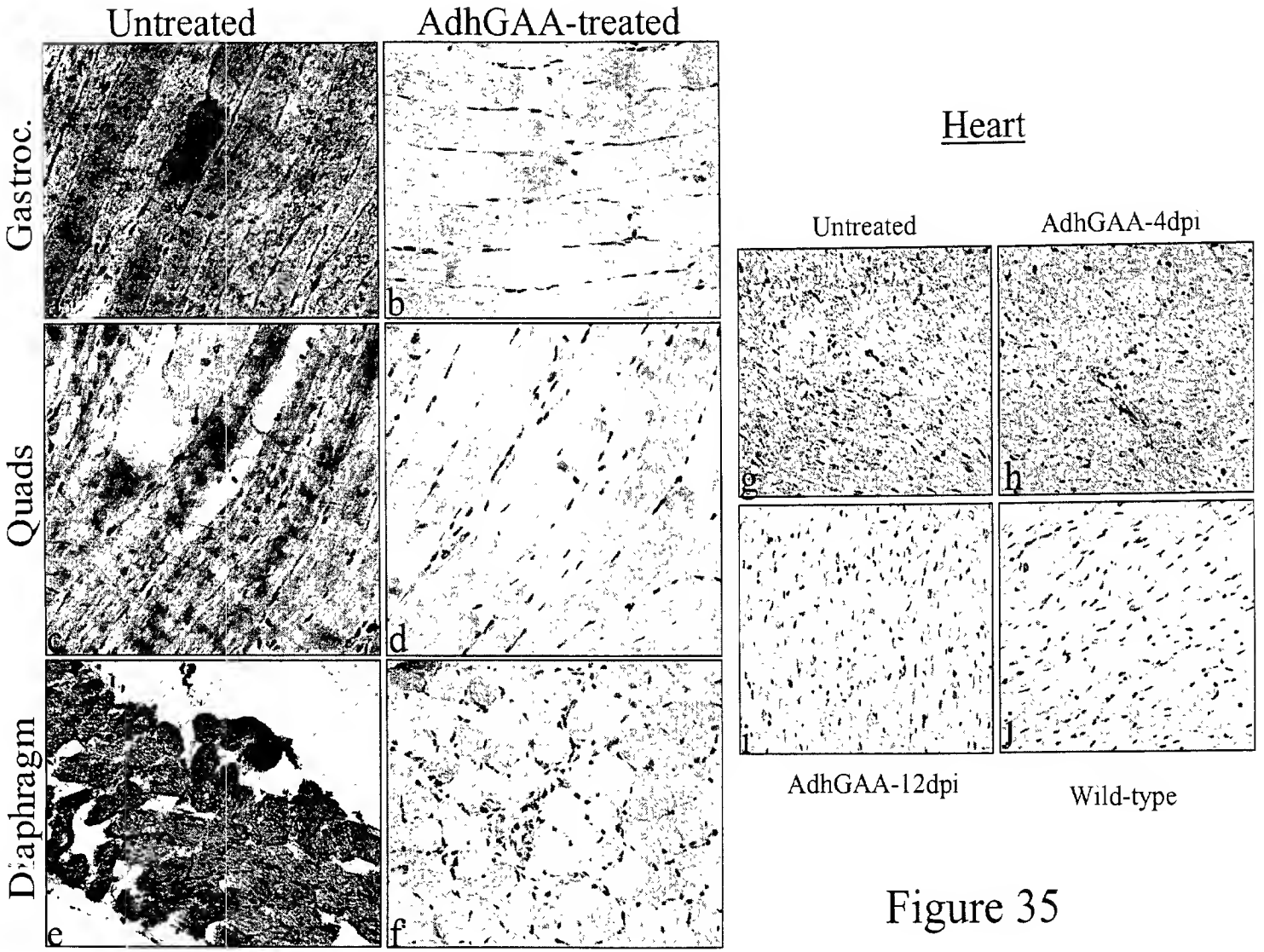
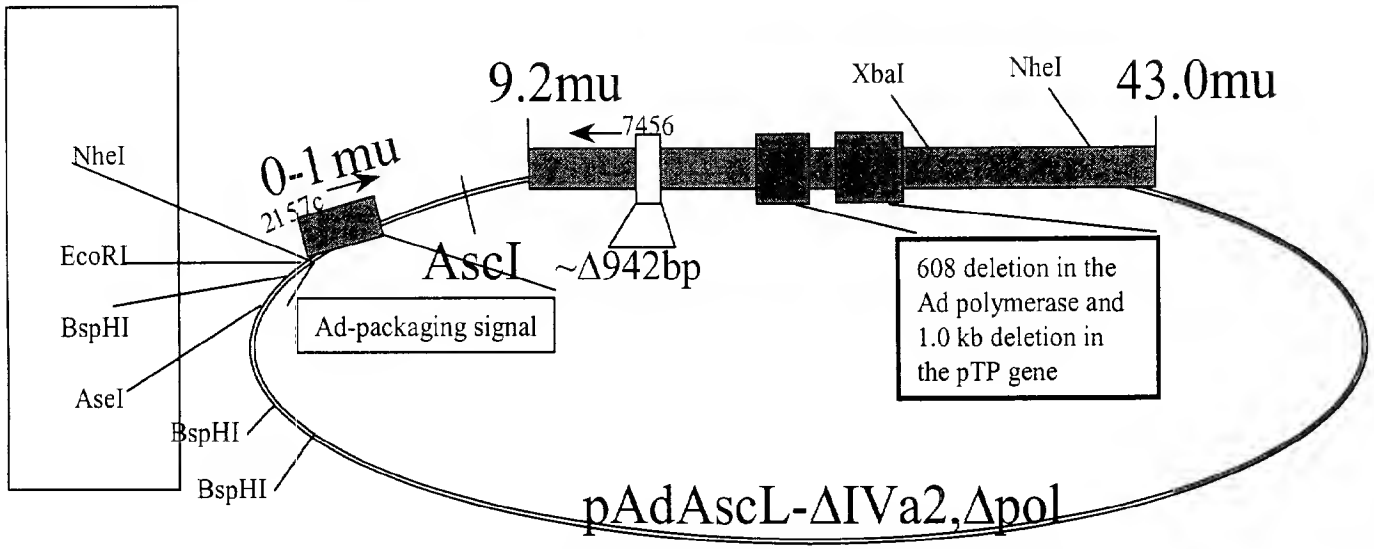

Untreated
AdhGAA-treated
Gastroc.
Quads
Diaphragm
a
b
c
d
e
f
Heart
Untreated
AdhGAA-4dpi
g
h
i
j
AdhGAA-12dpi
Wild-type

Figure 35

pAdAscL-ΔIVa2,Δpp-1.6kb:intermediate shuttle plasmid(~12.6kb) Amp-Res

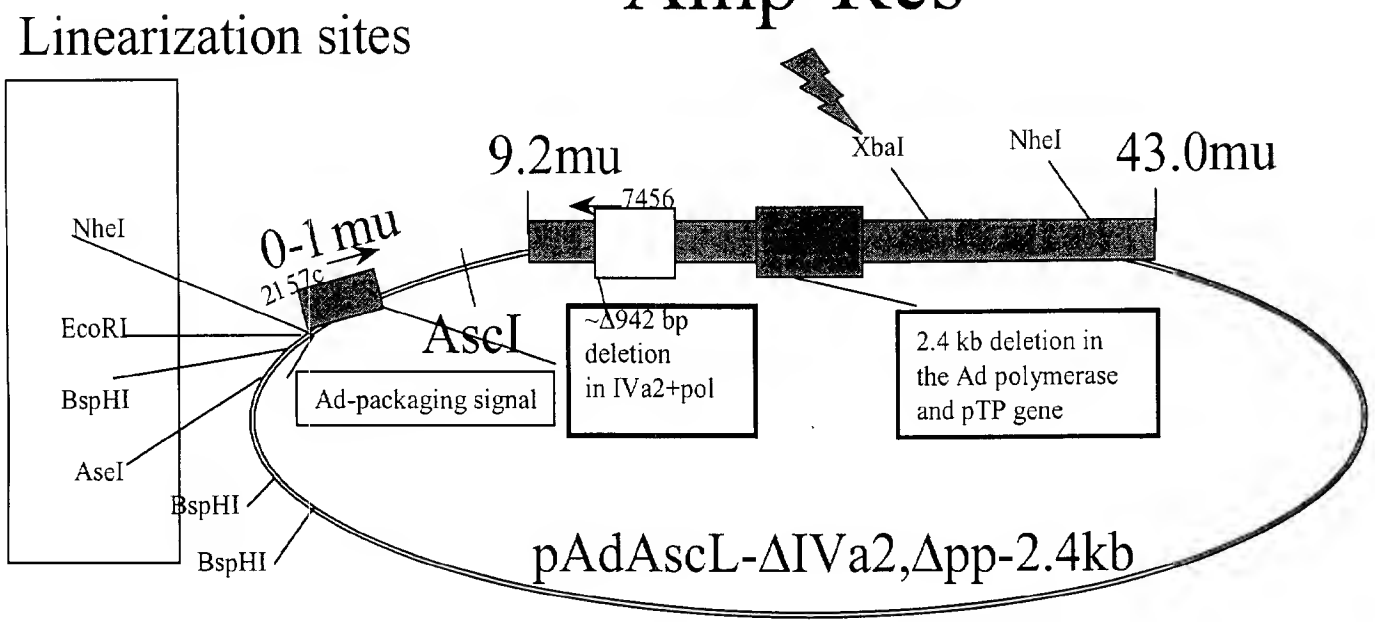
Linearization sites



← primer site location of indicated primer

Figure 36

pAdAscL-ΔIVa2,Δpp-2.4kb shuttle plasmid(~11.8kb) Amp-Res

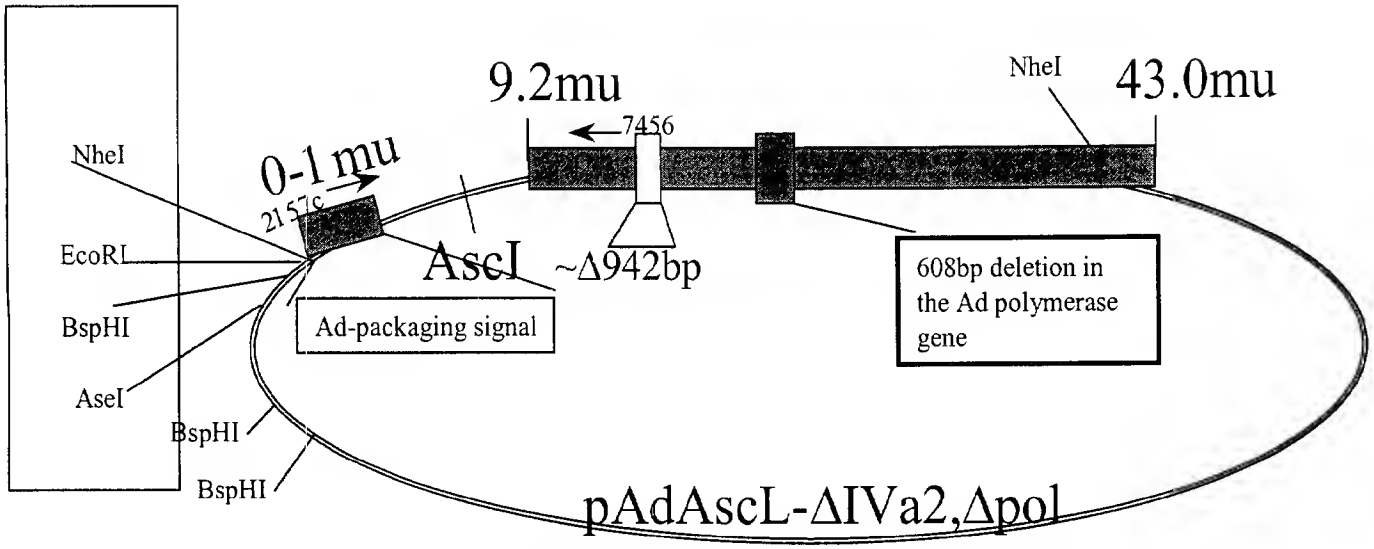


← primer site location of indicated primer

Figure 37

pAdAscL-ΔIVa2,Δpol shuttle plasmid(~13.6kb) Amp-Res

Linearization sites



← primer site location of indicated primer

Figure 38